Factors associated with the emergence of K65R in patients with HIV-1 infection treated with combination antiretroviral therapy containing tenofovir

HIV Cohort Study, Switzerland ; von Wyl, V ; Yerly, S ; Klimkait, T ; Böni, J ; Bürgisser, P ; Battegay, M ; Bernasconi, E ; Cavassini, M ; Furrer, H ; Hirschel, B ; Vernazza, P L ; Rickenbach, M ; Ledergerber, B ; Guenthard, H F

Abstract: BACKGROUND: The human immunodeficiency virus type 1 reverse-transcriptase mutation K65R is a single-point mutation that has become more frequent after increased use of tenofovir disoproxil fumarate (TDF). We aimed to identify predictors for the emergence of K65R, using clinical data and genotypic resistance tests from the Swiss HIV Cohort Study. METHODS: A total of 222 patients with genotypic resistance tests performed while receiving treatment with TDF-containing regimens were stratified by detectability of K65R (K65R group, 42 patients; undetected K65R group, 180 patients). Patient characteristics at start of that treatment were analyzed. RESULTS: In an adjusted logistic regression, TDF treatment with nonnucleoside reverse-transcriptase inhibitors and/or didanosine was associated with the emergence of K65R, whereas the presence of any of the thymidine analogue mutations D67N, K70R, T215F, or K219E/Q was protective. The previously undescribed mutational pattern K65R/G190S/Y181C was observed in 6 of 21 patients treated with efavirenz and TDF. Salvage therapy after TDF treatment was started for 36 patients with K65R and for 118 patients from the wild-type group. Proportions of patients attaining human immunodeficiency virus type 1 loads <50 copies/mL after 24 weeks of continuous treatment were similar for the K65R group (44.1%; 95% confidence interval, 27.2%-62.1%) and the wild-type group (51.9%; 95% confidence interval, 42.0%-61.6%). CONCLUSIONS: In settings where thymidine analogue mutations are less likely to be present, such as at start of first-line therapy or after extended treatment interruptions, combinations of TDF with other K65R-inducing components or with efavirenz or nevirapine may carry an enhanced risk of the emergence of K65R. The finding of a distinct mutational pattern selected by treatment with TDF and efavirenz suggests a potential fitness interaction between K65R and nonnucleoside reverse-transcriptase inhibitor-induced mutations.

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Factors associated with the emergence of K65R in HIV-1 infected patients treated with combination antiretroviral therapy containing tenofovir

Viktor von Wyl*, Sabine Yerly*, Jürg Böni, Philippe Burgisser, Thomas Klimkait, Manuel Battegay, Enos Bernasconi, Matthias Cavassini, Hansjakob Furrer, Bernard Hirschel, Pietro L. Vernazza, Martin Rickenbach, Bruno Ledergerber, Huldrych F. Günthard and the Swiss HIV Cohort Study

* contributed equally to this work

Corresponding Author:

Viktor von Wyl

Division of Infectious Diseases and Hospital Epidemiology

University Hospital Zurich

Raemistrasse 100

CH-8091 Zurich, Switzerland

Phone: ++41 (0)44 255 9660

Fax: ++41 (0)44 255 3291

vowv@usz.uzh.ch

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Author Affiliations:

Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich (Mr von Wyl, Drs Ledergerber and Günthard); Laboratory of Virology and AIDS Center, Geneva University Hospital, Geneva (Ms Yerly); National Center for Retroviruses, University of Zurich, Zurich (Dr Böni); Division of Immunology, Lausanne University Hospital, Lausanne (Dr Bürgisser); Institute for Medical Microbiology, University of Basel, Basel (Dr Klimkait); Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel (Dr Battegay); Division of Infectious Diseases, University Hospital Berne, Berne (Dr Furrer); University Hospital, University of Lausanne, Lausanne (Dr Cavassini); Division of Infectious Diseases, Geneva University Hospital, Geneva (Dr Hirschel); Division of Infectious Diseases, Cantonal Hospital St. Gallen, St. Gallen (Dr Vernazza); Division of Infectious Diseases, Regional Hospital Lugano, Lugano (Dr Bernasconi); Swiss HIV Cohort Study Data Center, Lausanne (Dr Rickenbach); Switzerland.

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Background

The HIV-1 reverse transcriptase mutation K65R is a single-point mutation, which has become more frequent after increased use of tenovofir (TDF). We aimed at identifying predictors for the emergence of K65R using clinical data and genotypic resistance tests (GRT) from the Swiss HIV Cohort Study.

Methods

222 patients with GRTs performed while being treated with TDF-containing regimens were stratified by detectability of K65R (K65R group, n=42 vs undetectable n=180). Characteristics at start of that treatment were analyzed.

Results

In an adjusted logistic regression, TDF-treatment with non-nucleoside reverse transcriptase inhibitors (NNRTI) and/or didanosine was associated with the emergence of K65R, whereas the presence of any of the thymidine analogue mutations (TAM) D67N, K70R, T215F, K219E/Q was protective. The previously undescribed mutational pattern K65R/G190S/Y181C was observed in 6 of 21 patients treated with efavirenz (EFV) and TDF. Salvage therapy following TDF treatment was started in 36 patients with K65R and in 118 of the wild type group. Proportions of patients attaining HIV-1 viral loads <50 copies/mL after 24 weeks of continuous treatment were similar between the K65R (44.1% [95%CI 27.2-62.1]) and the wild type group (51.9% [42.0-61.6]).

Conclusions

In settings where TAMs are less likely to be present, such as at start of firstline therapy or after extended treatment interruptions, combinations of TDF with other K65R-inducing components or with efavirenz or nevirapine may carry an enhanced risk for the emergence of K65R. The finding of a distinct mutational pattern favoured by treatment with TDF and EFV potentially suggests a fitness interaction between K65R and NNRTI-induced mutations.

Introduction

The nucleoside reverse transcriptase inhibitor (NRTI) tenofovir disoproxil fumarate (TDF) has become an important component of HIV combination therapy in Switzerland because of its potency and once-daily dosing [1, 2]. However, emergence of resistance and viral breakthrough can occur quickly, such as when TDF is used in combination with didanosine (ddI) and efavirenz (EFV)[3-5] or abacavir (ABC) and lamivudine (3TC)[6]. The key mutation for resistance against TDF is a switch of lysine to arginine at position 65 in the reverse transcriptase (RT) gene (K65R), which requires only one nucleotide base change [7, 8]. But contrary to other single point mutations inducing HIV drug resistance such as the RT mutation M184V, the prevalence of K65R in TDF-exposed individuals is limited , rarely exceeding 2% despite the widespread use of TDF and other drugs such as ABC and ddI [9, 10] which also select for K65R. Some increases in prevalence of K65R may, however, have occurred in recent years [11, 12].

Thymidine analogue mutations (TAM) favoured by zidovudine (AZT) or stavudine (d4T) counteract the selection of the K65R mutation, as shown both in vitro [13] and in patients [10, 12, 14, 15]. Parikh et al. have elucidated the biochemical mechanisms [16] and further demonstrated that TAMs and K65R do not appear on the same viral genome due to competing mutational pathways[17]. In contrast, inclusion of TDF in firstline therapy [2, 18, 19] or combination therapy with TDF and ddI [14, 20] promote the emergence of K65R. Our aim was to confirm and extend current knowledge on baseline predictors for the K65R mutation and to identify mutational correlates.

Methods

Data and patient selection

Our analysis included clinical and genotypic data collected up to July 2007. The SHCS is a nationwide, clinic-based cohort study with continuous enrolment and semi-annual study visits [21]. The SHCS has been approved by ethical committees of all participating institutions and written informed consent has been obtained from participants. The SHCS resistance database contains all

genotypic HIV resistance tests performed by the four authorized laboratories in Switzerland, stored in SmartGene's (Zug, Switzerland) Integrated Database Network System (IDNS version 3.4.0) [22]. The database was screened for resistance tests performed between January 2002 and July 2007 in patients on treatment with TDF or up to 30 days after treatment stop. Since tests were obtained under various circumstances (e.g. at therapy initiation or in salvage settings) we further restricted selection to reduce confounding. First, we excluded samples from patients who had previously experienced a virological failure on TDF, ABC, or ddI without resistance testing, since the K65R mutation may have already emerged in those patients. Moreover, we included only resistance tests which had been performed after ample exposure to TDF to allow for selection of the K65R mutation, which can occur as early as after 12 weeks of treatment [3, 6, 23]. Thus, we only considered tests which were performed after at least 90 days of continuous therapy with TDF or, in cases where patients already had prior exposure to TDF, tests done after 30 days of continuous treatment with the current regimen and at least 90 days of prior cumulative treatment with TDF. Only the first test per patient fulfilling all inclusion criteria was considered.

Throughout this project, virological failure was defined as an on-treatment HIV RNA level >500 copies/mL after at least 180 days of continuous treatment. Moreover, the study baseline was set at the start of the TDF-containing regimen for which a genotypic test was available, which did not necessarily correspond with the initiation of TDF.

Furthermore, we retrieved all available resistance tests conducted prior to the study baseline for included patients. The resistance database was complemented by retrospective sequencing of virus from frozen plasma samples in the SHCS repository (full protease gene and codons 29 to 225 of the RT gene) [24]. For this, plasma specimens with a viral load greater than 250 copies/mL were selected according to a predefined algorithm. Initially we searched for specimens obtained while the participant was on treatment with TDF, ddI or ABC. If none were available we further considered plasma samples taken near the latest virological failure events prior to study baseline; for the remaining patients we obtained pre-treatment specimens.

Analysis

Patients were grouped according to the presence or absence of the K65R mutation. Using the Mann-Whitney U test for continuous and Fisher's exact test for categorical variables, as well as univariable and multivariable logistic regression models, the following factors at start of the TDF treatment were compared between the two groups: socio-demographic characteristics; presence of TAMs, M184V, protease inhibitor (PI-) or non-nucleoside reverse transcriptase (NNRTI-) mutations; HIV-1 subtype; previous exposure to ddI, ABC or TDF; number of previous regimens; number of previous virological failures; and current treatment with ddI, ABC, NNRTI, PI or thymidine analogues. In a secondary analysis we further included viral factors potentially linked to the presence or absence of TAMs (RT mutations 214L and 83K) [25-27].

Associations of K65R with other RT mutations from on-treatment tests were assessed using Fisher's exact test with adjustments for multiple testing (Benjamini-Hochberg, 0.05 False Discovery Rate) [28]. Mutations selected for analysis were based on the 2006 International AIDS Society-USA drug mutation list [29]. TAMs were stratified into group 1 (M41L, L210W, T215Y) or group 2 (D67N, K70R, T215F, K219E, K219Q).

We compared treatment response to the first therapy following treatment with TDF between the K65R and the wild-type group by calculating the group-wise proportion of individuals attaining an HIV RNA <50 copies/mL at week 12 or 24. If such salvage treatment lasted for less than 12 weeks the patient was included in the week 12 analysis but excluded from the week 24 analysis.

Statistical analyses were performed with Stata 10 SE (StataCorp, College Station, TX, USA). All tests of significance were two-sided and p-values <0.05 were considered statistically significant.

Results

Prevalence of K65R

By July 2007 the SHCS drug resistance database contained samples from 70 patients with the K65R mutation, corresponding to a cumulative prevalence of 2.2% amongst all SHCS participants with at least one genotypic resistance test (figure 1). We found no time-trend for the prevalence of K65R for

the period of 2002-2007 (Cochran-Armitage p = 0.1536, data not shown), although we noted a jump in prevalence from 0.7% in 2002 to 2.0% in 2003, which coincided with the registration of TDF in Switzerland. Among patients with a resistance test performed on TDF the prevalence of K65R was 10.1%.

Clinical and genotypic correlates at baseline with K65R

We included in this analysis 222 patients (42 in the K65R group and 180 in the wild type group). For 32 (14.4%) of those 222 patients, the treatment under consideration was their first antiretroviral therapy (table 1). A total of 71 (32.0%) patients had already been exposed to TDF during a previous treatment period without virological failure (median exposure time 5.7 months ([IQR 2.9-11.3]). Genotypic resistance tests performed prior to start of the TDF-containing regimen were available for 186/220 patients (36, 85.7% in K65R group, 152, 84.4% in wild type group). No K65R mutation was detected in those samples.

Amongst 36 patients in the K65R group with a genotypic resistance test prior to start of TDF, 1 (2.8%) harboured viruses with TAMs 2 compared to 30 (19.7%) in the wild type group (table 1). No such difference was observed for TAMS group 1, which were detected in 4 patients from the K65R group (11.1%) and 25 patients (16.4%) from the wild type group. Moreover, patients of the K65R group were more frequently on firstline therapy (28.6%) than in the wild type group (11.1%) and a higher proportion was on combination therapy containing ddI (59.5% vs. 37.8% in wild type group). Of note, no instance of K65R was observed in 25 patients who had received AZT or d4T along with TDF. Therapies are detailed in table 2. We identified strong associations of K65R with the additional drug class included in combination therapy besides NRTIs. No instance of K65R emergence was observed on treatments including PI. Conversely, 91% of patients in the K65R group were on a combination therapy with NNRTIs. Accordingly, use of NNRTI arose as the most predictive factor associated with the emergence of K65R in a multivariable logistic regression analysis (OR 23.6 [95% CI 7.3 to 76.3], table 3). Other associations observed in this model were being on firstline therapy (OR 3.6 [1.1 to

12.2]) or being on combination therapy containing ddI (OR 3.6 [1.3 to 9.9]). No association of K65R with HIV subtype C was observed [30, 31].

Other RT mutations associated with K65R

We performed two analyses for identifying mutational associations of RT mutations with K65R. First, we considered only the 222 genotypic tests obtained on TDF (cross-sectional approach) and later also all preceding resistance tests if available, assuming that all mutations ever detected prior to the start of the TDF-containing regimen would still be present at time of resistance testing on TDF (cumulative approach, data not shown). A comparison of these two approaches allowed us to draw conclusions about the viral evolution of HIV-1 within patients.

Based on unadjusted p-values <0.05 from the cross-sectional analysis, we identified four NNRTI mutations (L100I, K103N, G190S, Y181C) which were more frequently observed in the K65R group, and three TAMs (M41L, D67N, T215Y), which were much rarer or absent in the K65R group (table 4). After adjustment for multiple testing only T215Y, G190S and Y181C reached statistical significance. The latter two mutations together with K65R were identified as a distinct mutational pattern in six patients treated with EFV and TDF. The cumulative approach confirmed that G190S and Y181C were not present at study baseline and must have been co-selected with K65R (data not shown).

In a secondary analysis we investigated associations of grouped TAMs with K65R (TAM 1 or TAM 2), again using the cross-sectional and the cumulative method. In the cross-sectional analysis, TAMs 1 were found in 2 patients from the K65R and in 41 patients from the wild type group. In contrast, the cumulative approach showed that 5 in the K65R and 44 in the wild type group harboured viruses with TAM 1. TAM 2 were detected in 1 and 45 patients from the K65R and the wild type group with the cross-sectional method and 2 and 53 patients with the cumulative approach. Thus viruses of four patients with K65R had lost TAMs between the baseline sample and the detection of the K65R mutation (3 with TAMs 1 and 1 with TAMs 2). Three of these patients had extended treatment breaks ranging from 1.5 to 4.8 years prior to starting the TDF containing regimen. The fourth patient was also on a therapy break for one year, but then resumed treatment with d4T, ddI and nevirapine [NVP],

achieving viral suppression prior to switching to therapy with TDF. Taken together, these four patients demonstrated shifts toward wild type once selection pressure by antiretroviral drugs was removed.

Clinical outcomes of TDF containing regimens

We further looked at treatment outcomes of the TDF containing regimens and continuing drug histories (figure 1). As of the database closure for this analysis, 168 patients had stopped the TDF-containing regimen. Immunologic or virologic failure was cited as the reason for stopping in 112 patients: 33 patients (78.6%) of the K65R group and 79 (43.9%) of the wild type group. Antiretroviral therapy-related toxicities were reported as the cause for stopping the TDF containing regimen in 16 (8.9%) patients of the wild type group, and 1 patient of the K65R group died while being treated with TDF. All other stop reasons (6 in K65R and 39 in wild type group) were either unknown or not clearly specified.

In total, 154 patients switched to a new therapy (36 from the K65R group, 118 from the wild type group). In the K65R group, 31 patients switched to a PI, of which 26 ritonavir boosted and 23 in combination with AZT or d4T. Moreover, 4 patients switched to single class NRTI therapy with ABC, and 1 patient remained on NNRTI treatment but replaced TDF with a different NRTI. At week 12, 38.9% of the K65R group and 41.5% of the wild type group showed a virological response to those new treatments (figure 1, Fisher's Exact p = 0.848). At week 24, virological response was 44.1% for patients with K65R and 51.9% in the wild type group (Fisher's exact p=0.556). An intent-to-treat approach yielded similar results (data not shown). We repeated these analyses with logistic regression models adjusted for baseline HIV RNA, the inclusion of enfuvirtide~~,~~ and the number of active drugs in the new regimen with a genotypic sensitivity score smaller than 15 as calculated by the Stanford algorithm based on cumulative drug resistance information [32]. We found no evidence that patients harbouring viruses with the K65R mutations had a worse treatment outcome at week 12 (Odds Ratio 1.2 [95% Confidence Interval 0.50 to 2.70]) and 24 (OR 0.92 [0.40 to 2.12]) when compared to the wild type group.

Discussion

In 222 patients on a TDF containing antiretroviral treatment, combinations of this drug with NNRTIs and/or ddI were highly associated with the emergence of the K65R mutation. In contrast, not a single patient on TDF combined with PI or thymidine analogues harboured viruses with the K65R mutation. The presence of one or several mutations of the TAM 2 group (D67N, K70R, 215F, 219Q and 219E) at start of the TDF-containing regimen appeared to have a protective effect against the emergence of K65R. We noted in four patients who had lost TAMs due to extended therapy interruptions that K65R could still be selected in spite of the likely presence of TAMs in minor viral populations. However, in none of these patients the TAMs re-emerged, further supporting the hypothesis that TAMs and the K65R mutation cannot exist on the same genome [17].

We further observed a previously undescribed pattern of NNRTI mutations (G190S, Y181C) and K65R in EFV-treated patients. Those NNRTI-specific mutations were not present at start of combination therapy with TDF and EFV and must have been co-selected with K65R.

Moreover, we investigated therapy success of the subsequent treatment in patients who had stopped the TDF containing regimen and were switched to a new regimen. We did not observe statistically significant differences between the K65R group and the wild type group in the proportion of patients with HIV RNA <50 copies/mL after 12 and 24 weeks of continuous treatment. At week 24 approximately half of the patients reached plasma viremia levels < 50 copies/mL, a result which is in line with or even better than salvage trials before tipranavir, darunavir and raltegravir became widely available [33-37]. Taking due account of small sample size and short follow-up, this suggests that patients who harbour viruses with the K65R mutation are as likely to attain viral loads of less than 50 copies/mL as patients with K65WT.

This study provides support for a protective effect of TAMs, and in particular TAMs 2, against the emergence of K65R. It is thus not surprising that patients who initiate their first ART with combination therapy including TDF are at a higher risk for acquiring K65R on virological failure as the prevalence of transmitted resistance and TAMs remains at approximately 10% in Switzerland [38]. In contrast, patients with extended treatment histories and previous virological failure are more likely

to harbour viruses carrying TAMs [von Wyl and Günthard, unpublished data]. Why were there no TAMs at study baseline in the pre-treated patients from the K65R group?. Extended treatment interruptions might be one answer, as case reviews from four patients indicated. Furthermore, we investigated viral factors (RT mutations 214L and 83K), which have recently been linked with the absence of TAMs in pre-treated patients [25, 26], but the analysis was not conclusive.

We noted that the additional drugs besides TDF included in combination therapy might play an important role in the emergence of K65R. Combinations of TDF and ddI appear problematic, since ddI and TDF share the same mutational pathway for selection of K65R.

Moreover, while K65R was absent in patients treated with PI, the use of EFV or NVP was highly associated with the emergence of K65R. This may be due to synergistic fitness effects with NNRTI-induced mutations on the reverse transcriptase, as our observation of the previously unreported K65R/G190S/Y181C mutational pattern in 6 EFV-treated patients suggests. **Since G190S is associated with a high fitness cost relative to wild type [39], K65R and/or Y181C may compensate for this.** Such fitness synergies have been described between the NRTI mutation L74V and the NNRTI mutations K103N and L100I [40].

Alternatively, K65R may occur preferentially on treatment with EFV or NVP compared to PI regimens because only one mutation is required to confer full resistance against those NNRTIs, whereas PIs often retain residual activity against HIV-1 despite the presence of PI mutations. Thus in combination therapy with TDF and NNRTI, insufficient intra-cellular concentrations of combination therapy with TDF, can quickly lead to viral breakthrough and full NNRTI resistance, followed by the emergence of additional mutations [3, 23]. In line with this argument, patients with no previous virological failure who were treated with an NNRTI and TDF (n=53) generally had viruses with more resistance mutations (median 3 [IQR 1-4]) than patients (n=44) on combination therapy with TDF and a ritonavir-boosted PI (0 [0-1], data not shown).

As these data stem from a representative cohort study reflecting current clinical practice, we consider the conclusions as clinically relevant. The study has limitations, however. We have compared patients

with highly diverse treatment histories, with no randomisation and cannot therefore exclude unmeasured confounding factors. Patients on tenofovir had to have a genotypic drug resistance test available, implying a selection bias. However, during this study genotypic resistance testing in patients failing antiretroviral treatment was already clinical routine [33]. Time-point of genotypic testing might also have confounded our results, in particular for the baseline resistance testing. **Predefined stringent selection criteria likely have minimized the impact of this. We also noted no systematic effect of timing of resistance testing on our results (data not shown).**

Our findings suggest that optimal future treatment regimens should avoid combining EFV or NVP with TDF and further NRTI drugs that favour the selection of the K65R mutation, such as DDI or ABC. Furthermore, the highly protective effect of boosted PIs and the observed antagonistic effects of TAMs on the emergence of K65R, suggest a potentially pivotal role of combining thymidine analogues, boosted PIs and tenofovir in salvage situations. This strategy should be explored in prospective studies.

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Potential Conflicts of Interest

Ms Yerly has participated in advisory board of BMS and has received travel grants from GSK and MSD. Dr Klimkait served on advisory boards for Abbott, Bayer, BMS, and Roche. Dr Battegay is a consultant for Roche Pharma Switzerland and Boehringer Ingelheim Switzerland. Drs Furrer and Vernazza have participated in advisory boards of Abbott, GSK, BMS, Roche, Gilead, MSD, Boehringer-Ingelheim, and Tibotec (Dr Vernazza). The institution of Dr Furrer has received unrestricted educational grants from Abbott, GSK, BMS, Roche, Gilead, MSD, and Boehringer-Ingelheim. Dr Bernasconi has received travel grants and honoraria from Gilead, Roche, GSK, Pfizer, Boehringer Ingelheim, and Tibotec. Dr Ledergerber has received travel grants, grants, or honoraria from Abbott, Aventis, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck Sharp & Dohme,

Roche, and Tibotec. Dr Günthard has been an adviser and/or consultant for the following companies: GSK, Abbott, Novartis, Boehringer Ingelheim, Roche, Tibotec and BMS. Dr Günthard has received unrestricted research and educational grants from Roche, Abbott, BMS, GSK, and MSD. Mr von Wyl and Drs. Böni and Cavassini state no conflict of interest.

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Tables

Table 1: Characteristics at the start of the TDF-containing regimen (base line)

	K65R n=42	Wildtype n=180	p
Female	16 (38.1%)	58 (32.2%)	0.472
Age	40.5 [37-47]	41 [37-46.5]	0.759
Mode of HIV Acquisition			
Heterosexual	16 (38.1%)	74 (41.1%)	0.871
Intravenous drug use	10 (23.8%)	40 (22.2%)	
Male having sex with male	14 (33.3%)	61 (33.9%)	
Other	2 (4.8%)	5 (2.8%)	
Ethnicity			
White	33 (78.6%)	137 (76.1%)	0.656
Black	6 (14.3%)	34 (18.9%)	
Other	3 (7.1%)	9 (5%)	
HIV Subtype			
B	33 (78.6%)	134 (74.4%)	0.124
CRF01_AE	2 (4.8%)	1 (.6%)	
C	2 (4.8%)	8 (4.4%)	
Other	5 (11.9%)	37 (20.6%)	
Nadir CD4 (median [IQR]) [a]	132 [60-241]	142 [50-220]	0.980
Baseline CD4 (median [IQR]) [a]	191 [90-288]	266 [153-407]	0.016
Baseline log10 HIV RNA (median [IQR]) [a]	4.6 [2.3-5.3]	3.8 [1.2-5.2]	0.095
Previous CDC C event	30 (71.4%)	108 (60%)	0.216
Baseline mutations			
Baseline test available	36 (85.7%)	152 (84.4%)	1.000
TAMS (any)	5 (13.9%)	45 (29.6%)	0.061
TAMS Group 1 [b]	4 (11.1%) b	25 (16.4%)	0.608
TAMS Group 2 [c]	1 (2.8%) c	30 (19.7%)	0.011
NNRTI mutations	9 (25%)	30 (19.7%)	0.497

PI mutations	4 (11.1%)	31 (20.4%)	0.241
RT184V/I	11 (30.6%)	54 (35.5%)	0.698
RT214L	7 (19.4%)	36 (23.7%)	0.665
RT83K	10 (27.8%)	31 (20.4%)	0.371
On firstline antiretroviral therapy	12 (28.6%)	20 (11.1%)	0.007
Current Treatment: Tenovovir combined with			
NNRTI	38 (90.5%)	56 (31.1%)	0.000
PI	0 (0%)	108 (60%)	0.000
Didanosine	25 (59.5%)	68 (37.8%)	0.014
Lamivudine or emtricitabine	17 (40.5%)	99 (55%)	0.122
Abacavir	3 (7.1%)	18 (10%)	0.772
Zidovudine or stavudine	0 (0%)	25 (13.9%)	0.006
More than 95% adherent [d]	13 (41.9%)	82 (50%)	0.439
Treatment history [e]			
Number of previous regimens (median [IQR])	4.5 [3.0-7.0]	5.0 [3.0-7.0]	0.794
Previous exposure to didanosine or abacavir	17 (56.7%)	93 (58.1%)	0.882
Previous exposure to tenofovir	9 (30.0%)	62 (38.8%)	0.363
Number of previous virological failure			
0	16 (53.4%)	81 (50.6%)	0.745
1	10 (33.3%)	56 (35.0%)	
2 or more	4 (13.3%)	23 (14.4%)	
Virological failure on zidovudine or stavudine	13 (43.3%)	80 (50.0%)	0.503
Duration of previous exposure to zidovudine or stavudine in years (median [IQR])	2.7 [1.1-5.3]	3.6 [1.0-6.3]	0.364
Virological failure on NNRTI	2 (6.7%)	15 (9.4%)	0.633
Virological failure on lamivudine	11 (36.7%)	73 (45.6%)	0.365

Numbers represent n (%) unless otherwise stated.

Abbreviations: TAMs, thymidine anlalogue mutations; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RT, reverse transcriptase.

[a] Baseline laboratory parameters available for 34 and 179 of the K65R and the wild type group, respectively.

[b] TAMs group 1: any RT gene mutation of the following: 41L, 210W, 215Y. 3 of 4 present as mixture in K65R group.

[c] TAMs group 2: any RT gene mutation of the following: 67N, 70R, 215F, 219E, 219Q. Present as mixture in K65R group.

[d] Adherence measure only available for 31 and 164 of the K65R and the wild type group, respectively.

[e] Comparison only for patients not on firstline therapy (30 in K65R group and 160 in wild type group).

Table 2: Antiretroviral therapy combinations with tenofovir. The denominator is the number of patients on a specific regimen, stratified by the two treatment groups (patients on firstline therapy and patients on later treatments).

	Firstline regimens		Later regimens	
Combinations including tenofovir	**K65R**	**Wildtype**	**K65R**	**Wildtype**
Efavirenz and lamivudine/emtricitabine	5 (36%)	9 (64%)	2 (17%)	10 (83%)
Efavirenz and didanosine	0	0	13 (46%)	15 (54%)
Efavirenz and abacavir	0	0	1 (100%)	0
Nevirapine and lamivudine/emtricitabine	4 (100%)	0	3 (60%)	2 (40%)
Nevirapine and didanosine	3 (100%)	0	7 (54%)	6 (46%)
Boosted atazanavir and lamivudine/emtricitabine	0	0	0	18 (100%)
Lopinavir and lamivudine/emtricitabine	0	6 (100%)	0	10 (100%)
Lopinavir and didanosine	0	1 (100%)	0	12 (100%)
Other NNRTI and NRTI	0	0	0	7 (100%)
Other boosted PI and NRTI	0	0	0	36 (100%)
Other unboosted PI and NRTI	0	0	0	13 (100%)
Three class combination (PI, NNRTI, NRTI)	0	0	0	4 (100%)
Single class NRTI	0	4 (100%)	4 (17%)	20 (83%)
Other	0	0	0	7 (100%)

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 3: Factors associated with the presence of K65R in univariable and multivariable logistic regressions (n=222).

	Univariable		Multivariable	
Variables	**Odds Ratio**	**95% CI**	**Odds Ratio**	**95% CI**
Baseline Mutations				
TAMs Group 1 present [a]	0.63	[0.21 to 1.95]	1.11	[0.20 to 6.03]
No TAMs Group 1 present	1	[Reference]	1	[Reference]
No baseline resistance test	0.85	[0.32 to 2.23]	0.79	[0.19 to 3.33]
TAMs Group 2 present [b]	**0.12**	**[0.02 to 0.92]**	**0.07**	**[0.01 to 0.64]**
No TAMs Group 1 present	1	[Reference]	1	[Reference]
No baseline resistance test	0.75	[0.29 to 1.96]	Not done [c]	
NNRTI mutations present	1.36	[0.58 to 3.18]		
No NNRTI mutations present	1	[Reference]		
No baseline resistance test	0.97	[0.37 to 2.57]		
PI mutations present	0.49	[0.16 to 1.48]		
No PI mutations present	1	[Reference]		
No baseline resistance test	0.81	[0.31 to 2.12]		
Female	1.29	[0.64 to 2.60]	2.43	[0.71 to 8.33]
Age (per year increase)	1.00	[0.97 to 1.04]	1.02	[0.97 to 1.07]
Mode of HIV Acquisition				
Heterosexual contact	1	[Reference]	1	[Reference]
Injection drug use	1.16	[0.48 to 2.78]	3.08	[0.80 to 11.89]
Homosexual-bisexual contact	1.06	[0.48 to 2.35]	2.30	[0.60 to 8.74]
Other	1.85	[0.33 to 10.40]	0.66	[0.06 to 7.25]
Previous CDC stage C event	1.67	[0.80 to 3.47]	**2.87**	**[1.05 to 7.84]**

Baseline CD4 count (per 10 cells increase)	0.85	[0.71 to 1.02]		
Baseline log10 HIV RNA (per log increase)	1.14	[0.97 to 1.35]		
Ethnicity				
White	1	[Reference]	1	[Reference]
Black	0.73	[0.28 to 1.89]	0.96	[0.15 to 6.18]
Other	1.38	[0.35 to 5.40]	0.74	[0.08 to 7.12]
HIV Subtype				
B	1	[Reference]		
CRF01_AE	8.12	[0.71 to 92.29]	27.08	[0.57 to 1,282]
C	1.02	[0.21 to 5.01]	0.94	[0.09 to 9.45]
Other	0.55	[0.20 to 1.50]	0.64	[0.10 to 4.15]
Adherence				
Adherence <95%	1.38	[0.64 to 3.01]		
Adherence >=95%	1	[Reference]		
No information available	**4.34**	**[1.65 to 11.39]**		
On firstline antiretroviral therapy	**3.20**	**[1.42 to 7.23]**	**3.64**	**[1.08 to 12.24]**
Current Treatment: Tenovovir combined with				
Zidovudine or stavudine [d]	**0.11**	**[0 to 0.62]**	Not done	
Lamivudine	0.56	[0.28 to 1.10]		
Abacavir	0.69	[0.19 to 2.47]		
Didanosine	**2.42**	**[1.22 to 4.81]**	**3.62**	**[1.32 to 9.94]**
NNRTI	**21.04**	**[7.16 to 61.79]**	**23.59**	**[7.29 to 76.28]**
PI [d]	**0.01**	**[0 to 0.06]**	Not done	
Previous exposure to didonasine or abacavir	0.64	[0.32 to 1.26]		
Previous exposure to tenofovir	0.52	[0.23 to 1.15]		
Previous failure events				
No previous virological failure	1	[Reference]		
1 previous virological failure	0.64	[0.29 to 1.42]		
2 or more previous virological failures	0.63	[0.20 to 1.96]		

Previous virological failure on lamivudine	0.52	[0.25 to 1.10]
Previous virological failure on NNRTI	0.55	[0.12 to 2.50]
Previous virological failure on zidovudine or stavudine	0.56	[0.27 to 1.15]

Abbreviations: TAMs, thymidine anlalogue mutations; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RT, reverse transcriptase.

a TAMs group 1: any RT gene mutation of the following: 41L, 210W, 215Y.

b TAMs group 2: any RT gene mutation of the following: 67N, 70R, 215F, 219E, 219Q.

c Not included in multivariable model because of collinearity.

d Odds ratio from exact logistic regression. For computational reasons only univariable estimates could be obtained.

Table 4: Reverse transcriptase mutations associated with K65R in genotypic resistance tests performed on combination therapy with tenofovir. Denominators are the number of patients in each group ever exposed to the respective drug class (NNRTI, thymidine analogues, NRTIs without thymidine analogues).

Mutation type	Mutation	K65R	Wildtype	P-value [a]	Critical p [b]	Significant
NNRTI						
	100I	6/40 (15%)	5/137 (3.6%)	0.018	0.009	
	103N	16/40 (40%)	31/137 (22.6%)	0.041	0.014	
	106M	2/40 (5%)	2/137 (1.5%)	0.220	0.027	
	106A	1/40 (2.5%)	0/137 (0%)	0.226	0.029	
	108I	3/40 (7.5%)	7/137 (5.1%)	0.696	0.042	
	181C [c]	19/40 (47.5%)	7/137 (5.1%)	0.000	0.002	yes
	188L	1/40 (2.5%)	4/137 (2.9%)	1.000	0.044	
	188C	2/40 (5%)	1/137 (.7%)	0.128	0.018	
	188H	0/40 (0%)	2/137 (1.5%)	1.000	0.045	
	190A	6/40 (15%)	8/137 (5.8%)	0.090	0.015	
	190S	8/40 (20%)	2/137 (1.5%)	<0.001	0.003	yes
	190E	1/40 (2.5%)	1/137 (.7%)	0.402	0.030	
	225H	1/40 (2.5%)	1/137 (.7%)	0.402	0.032	
TAMs group 1						
	41L	1/30 (3.3%)	32/155 (20.6%)	0.020	0.011	
	210W	0/30 (0%)	15/155 (9.7%)	0.136	0.020	
	215Y	0/30 (0%)	29/155 (18.7%)	0.005	0.006	yes
	Any	1/30 (3.3%)	37/155 (23.9%)	0.012	0.008	
TAMs group 2						
	67N	1/30 (3.3%)	31/155 (20%)	0.032	0.012	
	70R	1/30 (3.3%)	20/155 (12.9%)	0.207	0.023	

215F	0/30 (0%)	12/155 (7.7%)	0.220	0.024	
219Q	0/30 (0%)	12/155 (7.7%)	0.220	0.026	
219E	0/30 (0%)	6/155 (3.9%)	0.592	0.035	
Any	1/30 (3.3%)	42/155 (27.1%)	0.004	0.005	yes
NRTI (other than TAMs)					
115F	2/42 (4.8%)	1/180 (.6%)	0.093	0.017	
116Y	0/42 (0%)	1/180 (.6%)	1.000	0.047	
70E [d]	2/42 (4.8%)	7/180 (3.9%)	0.680	0.039	
151M	0/42 (0%)	1/180 (.6%)	1.000	0.048	
62V	2/42 (4.8%)	2/180 (1.1%)	0.163	0.021	
74V	2/42 (4.8%)	7/180 (3.9%)	0.680	0.041	
184V/I	14/40 (35%)	59/168 (35.1%)	1.000	0.050	
Other (not drug resistance related)					
214L	7/42 (16.7%)	38/180 (21.1%)	0.671	0.038	
68G	2/42 (4.8%)	5/180 (2.8%)	0.619	0.036	
83K	9/42 (21.4%)	30/180 (16.7%)	0.501	0.033	

Abbreviations: TAMs, thymidine anlalogue mutations; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; RT, reverse transcriptase.

[a] Fisher's Exact test.

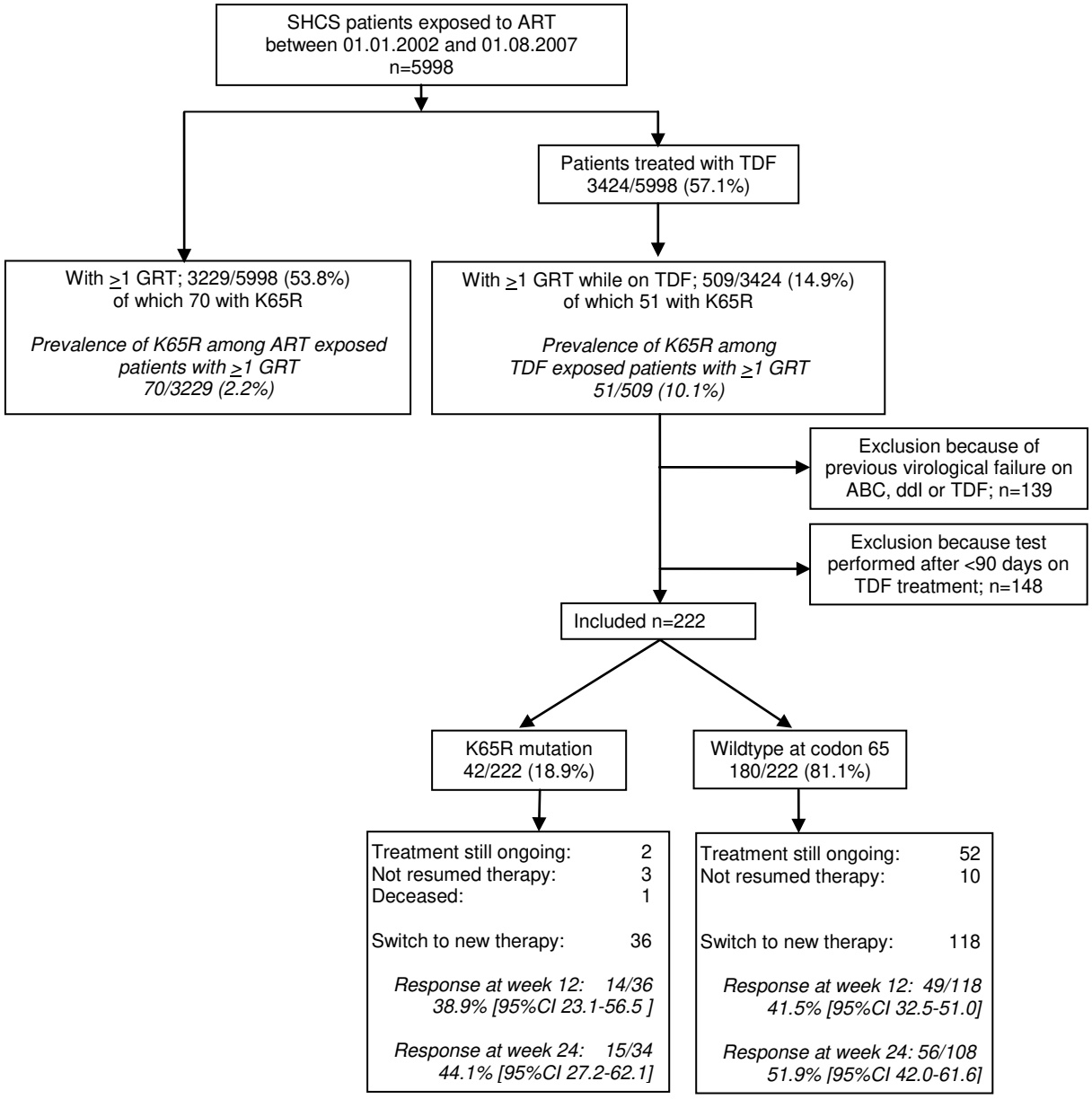
[b] Benjamini-Hochberg critical value, assuming a false discovery rate of 0.05.

[c] In 6 patients treated with efavirenz, a distinct mutational pattern consisting of G190S and Y181C was observed, which always appeared in combination with K65R. Those patients are all infected with subtype B viruses.

[d] In the two patients from the K65R group, K65R and K70E were present as mixtures.

Figures

Figure 1 Flow chart of patient selection and calculation of prevalence of K65R.



Abbreviations: ART, antiretroviral therapy; GRT, genotypic resistance test; TDF, tenofovir; ddI, didanosine; ABC, abacavir; 95%CI, 95% Confidence Interval.